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An Efficient Pharmacophore Generation Strategy To Identify New Leukotriene A4 Hydrolase (lta4h)

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Supporting Information

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Abstract

Leukotriene A4 Hydrolase as a potent inhibitor in inflammation. Leukotriene A4 hydrolase, also known as LTA4H is a human gene. The protein encoded by this gene, a bifunctional zinc enzyme converts leukotriene A4 to leukotriene B4 (LTB4). Arachidonic acid is a polyunsaturated fatty acid that is present in the phospholipids of membranes of the body's cells, and is abundant in the brain. It is involved in cellular signaling as a lipid second messenger. LTA4H may play an important role in carcinogenesis, especially chronic inflammation-associated carcinogenesis by two ways.

a) The inflammation-augmenting effect of inflammatory cells through positive

b) The autocrine growth-stimulatory effect of LTB4 produced by epithelial cells, and the paracrine growthstimulatory effect of LTB4 produced by inflammatory cells, on precancerous and cancer cells.

Many drugs like Bestatin (N-[(2S,3R)-3-amino-2-hydroxy-4-phenyl-butanoyl]-L-leucine), Thioamine (3-(4-benzyloxyphenyl)-2-(R)-amino-1-propane thiol) Hydroxamicacid((N-hydroxy-N-[(2S)-2-amino-3-(benzyloxyphenyl)propyl]-5-carboxypentanamide) are available in the market which inhibits hydrolase activity. The variability in potency found in LTA4H inhibitors (50% inhibitory concentrations (IC50s) ranging from submicromolar to high-micr-omolar values for structurally related molecules) suggests that accessory binding pockets in the enzyme active site must be present and play a pivotal, still unclarified, role in determining the affinity. It is therefore conceivable that the identification of such areas can be exploited for the design of novel, more potent LTA4H inhibitors.

In view of the potential therapeutic importance of LTA4H inhibitors, we engaged ourselves in a program devoted to the design feature based three-dimensional pharmacophore model for LTA4H inhibitors. In this frame, we report here a molecular modeling study aimed at mapping the topography of the active site of LTA4H and at identifying the structural requirement for LTA4H inhibition. Many LTA4H inhibitors for which homogeneous biological values are available were collected from the literature along with their inhibitory IC50 values. The Generated model can be further utilized for developing new potentially active candidates targeting LTA4H, which can be useful as anti inflammation agents.

Introduction

Based on the resolution and the interaction of the self ligand in the protein hydrolase and zinc domain part, the PDB of 2VJ8 (with the resolution of 1.80 Å) has been selected for the study, all the molecules were built using builder module of Cerius2[7] and minimized using the steepest descent algorithm with a convergence gradient value of 0.001 kcal/mol. Docking was done to analyze the ligand–protein interaction.

Pharmacophore Generation

For the pharmacophore modeling studies, a set of LTA4H inhibitory activity data (IC_{50}) spanning over 4 orders of magnitude (from 1 to 630,000 nM) were selected. The dataset was divided into training set (22) Fig 1 and test set (168). The training was selected by considering diversity in The most active, several moderately active, and some inactive compounds were also included in order to obtain critical information on pharmacophore requirements The important aspect of this selection scheme was that each active compound would teach something new to the HypoGen module to help it uncover as much critical information as possible for predicting biological activity. To generate 3D pharmacophore, each compound should have conformations to cover three dimensional space. For this, conformational models of all molecules were generated using the ‘best quality’ conformational search option within the Catalyst’s ConFirm module. It generates the conformations using the ‘Poling’ algorithm. A maximum of 250 conformations were generated for each compound to ensure maximum coverage in the conformational space within an default energy threshold of 20.0 kcal/mol above the global energy minimum. Ten best Pharmacophore (called hypotheses in the program) models were generated using HypoGen module. An initial analysis revealed that four chemical feature types such as Hydrogen-bond Acceptor (HA),

Hydrogen-bond Donar(HD), Hydrophobic aliphatic (HPAli), Hydrophobic aromatic(HPAr) features could effectively map all critical chemical features of all molecules in the training and test sets. These features were selected and used to build a series of hypotheses using default uncertainty value 3 (defined by Catalyst as the measured value being within three times higher or three times lower of the true value). Indeed, Catalyst generates a chemical-feature-based model on the basis of the most active compounds. These compounds are determined by performing a simple calculation based on the activity and uncertainty. In hypothesis generation, the structure and activity correlations in the training set were rigorously examined. HypoGen identifies features that were common to the active compounds but excludes from the inactive compounds within conformationally allowable regions of space. It further estimates the activity of each training set compound using regression parameters. The parameters are computed by the regression analysis using the relationship of geometric fit value versus the negative logarithm of activity. The greater the geometric fit, the greater the activity prediction of the compound. The fit function does not only check if the feature is mapped or not, it also contains a distance term, which measures the distance that separates the feature on the molecule from the centroid of the hypothesis feature.

The generated pharmacophore model should be statistically significant, should predict activity of the molecules accurately, and should identify active compound from a database. Therefore, the derived pharmacophore map was validated using (1a) *Cost analysis*, (1b) *Test set prediction*.

(1a) Cost analysis: The HypoGen module in Catalyst performs two important theoretical cost calculations (represented in bit units) that determine the success of any pharmacophore hypothesis. One is the 'fixed cost' (also termed as ideal cost), which represents the simplest model that fits all data perfectly, and the second one is the 'null cost' (also termed as no correlation cost), which represents the highest cost of a pharmacophore with no features and estimates activity to be the average of the activity data of the training set molecules. A meaningful pharmacophore hypothesis may result when the difference between null and fixed cost value is large; a value of 40–60 bits for a pharmacophore hypothesis may indicate that it has 75–90% probability of correlating the data. The total cost (pharmacophore cost) of any pharmacophore hypothesis should be close to the fixed cost to provide any useful models.

Two other parameters that also determine the quality of any pharmacophore hypothesis with possible predictive values are the configuration cost or entropy cost, which depends on the complexity of the pharmacophore hypothesis space and should have a value

(1b) Test set activity prediction: In addition to estimation of activity of training set molecules, the pharmacophore model should also estimate the activity of new compounds Therefore, a large set of 168 LA4H inhibitors with wide range of activity and large chemical diversity (supporting information), which were not included in training set, was considered as a test set. These molecules are covering wide range of activities spanning from 1 to 6300000 nM. The best pharmacophore (Hypo 1) having high correlation coefficient (r), lowest total cost, and lower RMSD value was chosen to estimate the activity of test set.

Methods

The pharmacophore model has two hydrogen bond acceptor (HA), two Hydrogen-bond Donar(HD), and two Hydrophobic aromatic(HPAr), Fig 2 shows the best hypothesis model Hypo 1 produced by the HypoGen module in Catalyst 4.10 software Fig 3 shows the Hypo1 aligned with the highest active compound, and Fig 4 shows the Hypo1 aligned with the lowest active compound of the training set. Fig 5 shows the Hypo1 aligned with the highest active compound, and Fig 6 shows the Hypo1 aligned with the lowest active compound of the test set. Results of pharmacophore hypotheses are presented in table 1. The first hypothesis (Hypo1) was the best pharmacophore hypothesis, which is characterized by the highest cost difference (53.62), lowest root-mean-square error (1.0867), and the best correlation coefficient (0.92) shown in Fig 7. The fixed cost, and null cost are 91.1529 and 158.768 respectively. The generated pharmacophore model has predicted the activity of a large and diverse dataset of 168 test set compounds in table 2 with correlation of 0.86 shown in Fig 8. As we can see from Fig 3 & 5 , the compounds were predicted correctly. The features of the Hypo1 are fitting well to all the chemical features of highly active test set compounds .

Docking were performed using ligand fit program with the highest(Training set Compound 1) and least active(Training set Compound 22) compounds, the docking score of the above stated molecules are all positive values (Table 4). Highest active molecule (Training set Compound 1) which has been subjected to ligand fit showing its interactions with Gln 136, Gly

268, Gly 269, Glu 271, His 295, Glu 296 and His 299 amino acids of protein 2VJ8 shown in fig 9, with a docking score of 468.689 and Lowest active molecule (Training set Compound 22) which has been subjected to ligand fit showing its interaction with His 295, His 299 and Tyr 383 amino acids of the protein 2VJ8 shown in fig 10, with a docking score of 401.880.

See supporting information for more details.

Results

The work in the catalyst presented in the study shows how chemical features hydrogen acceptor, hydrophobic aliphatic of set of compounds along with their activities ranging over several orders of magnitude can be used to generate pharmacophore hypothesis, that can successfully predict the activity. The models were not only predictive within the same series of compounds but differences classes of diverse compounds also effectively mapped onto most of the features important for activity as they had a good correlation value of 0.92 and a good configuration 13.45. The pharmacophore generated can be used for diversified structures that can be potentially inhibit LTA4H inhibitors discovery and to evaluate how well any newly designed compound maps in the pharmacophore developed in this study, using inhibitors against LTA4H showed distinct features that may be responsible for the activity of the inhibitors.

Conclusion(s)

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Smellie,A.;Teig,S.L.;Towbin,P.J.Comput.Chem.1995 , 16 ,171.

Illustrations

Illustration 1

The best hypothesis model Hypo 1 produced for the inhibitor molecules of LTA4H protein

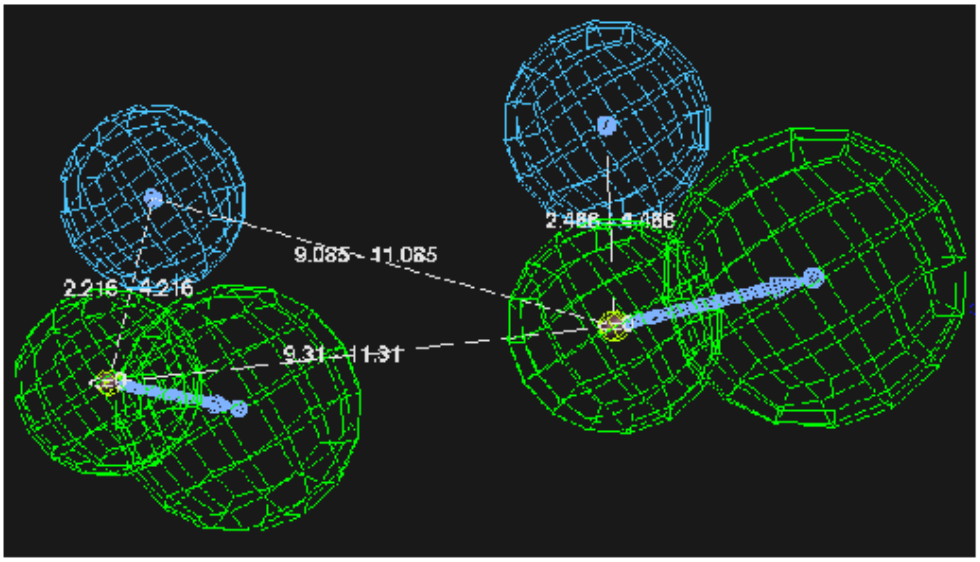

Illustration 2

Overlapping of highest active inhibitor molecule of trainingset with the best pharmacophore Hypo1.

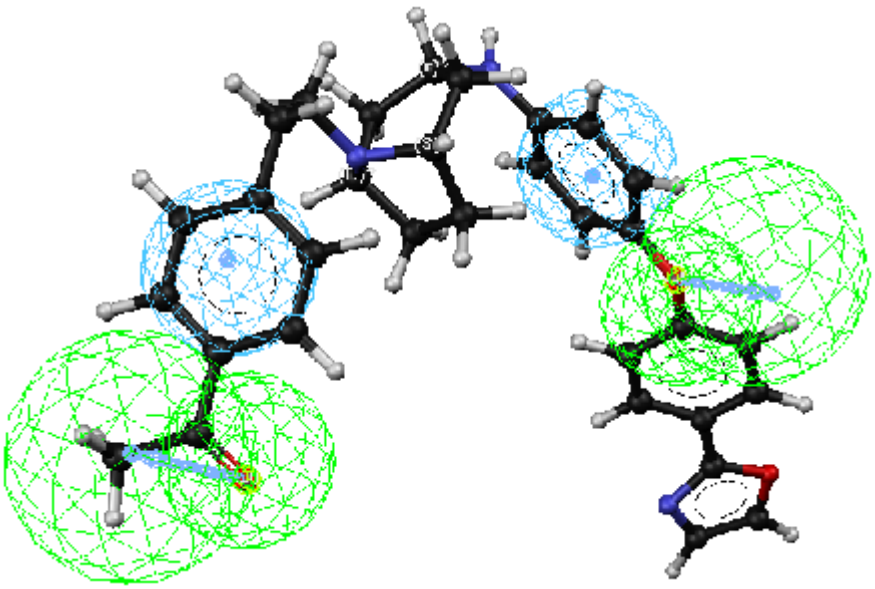

Illustration 3

Overlapping of lowest active inhibitor molecule of training set with the best pharmacophore Hypo1

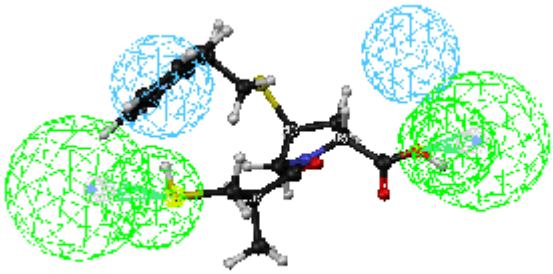

Illustration 4

Overlapping of highest active inhibitor molecules of test set with the best pharmacophore (Hypo1)

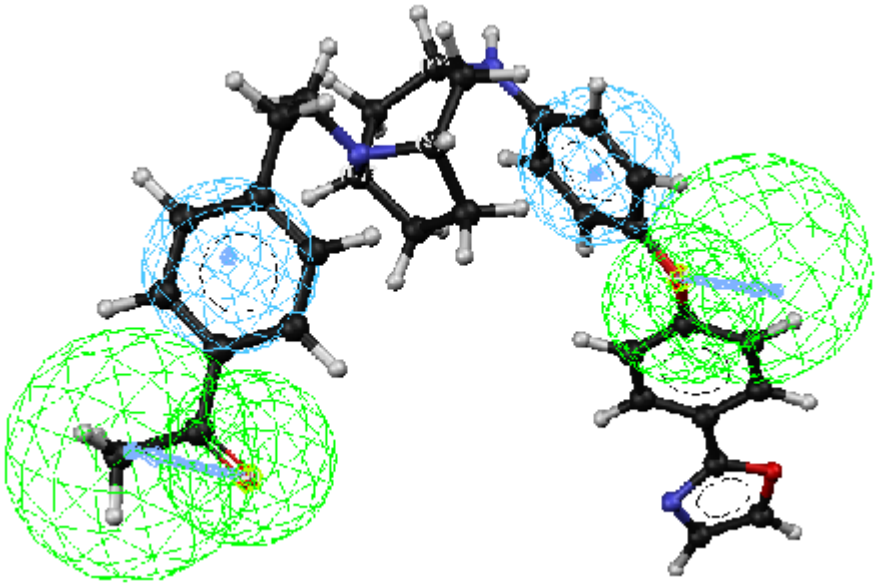

Illustration 5

Overlapping of lowest active inhibitor molecules of test set with the best pharmacophore (Hypo1)

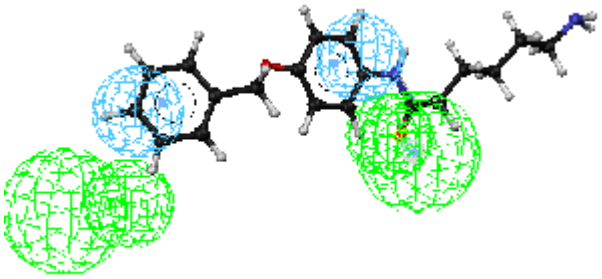

Illustration 6

Correlation coefficient of training set molecules is 0.94

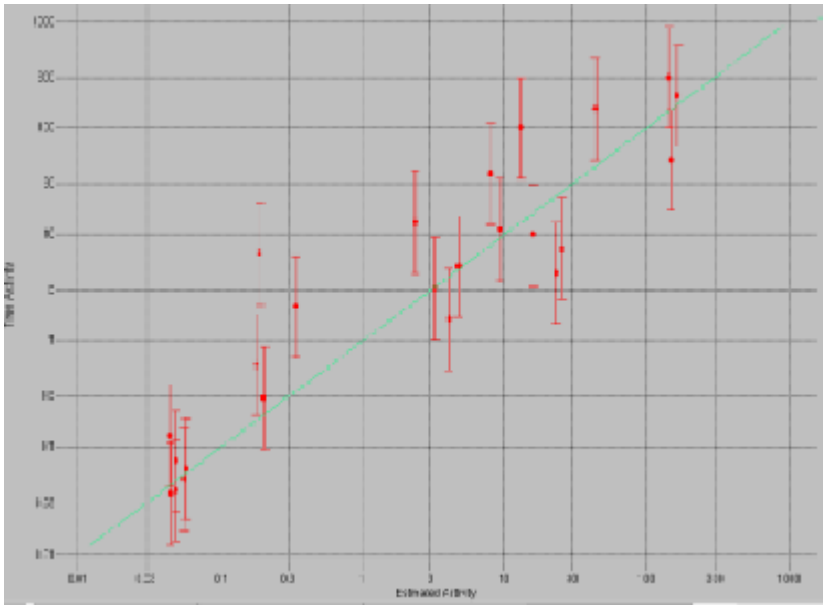

Illustration 7

Correlation graph between experimental and Hypo 1-estimated activities of test set

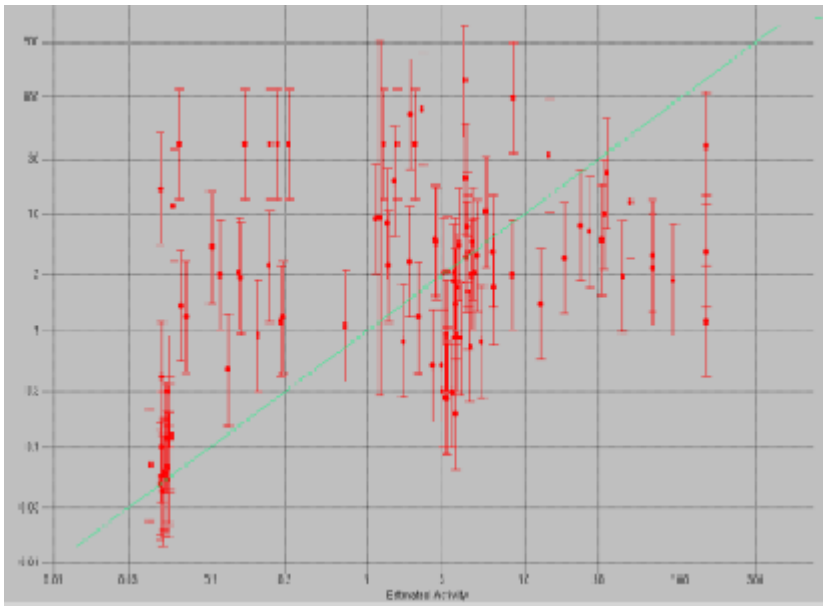

Illustration 8

Highest active molecule (Training set Compound 1) which has been subjected to ligand fit.

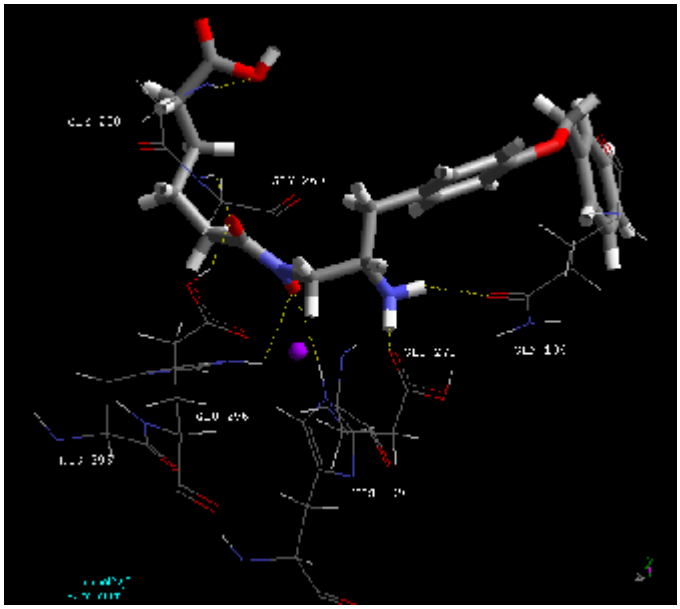

Illustration 9

Lowest active molecule (Training set Compound 22) which has been subjected to ligand fit.

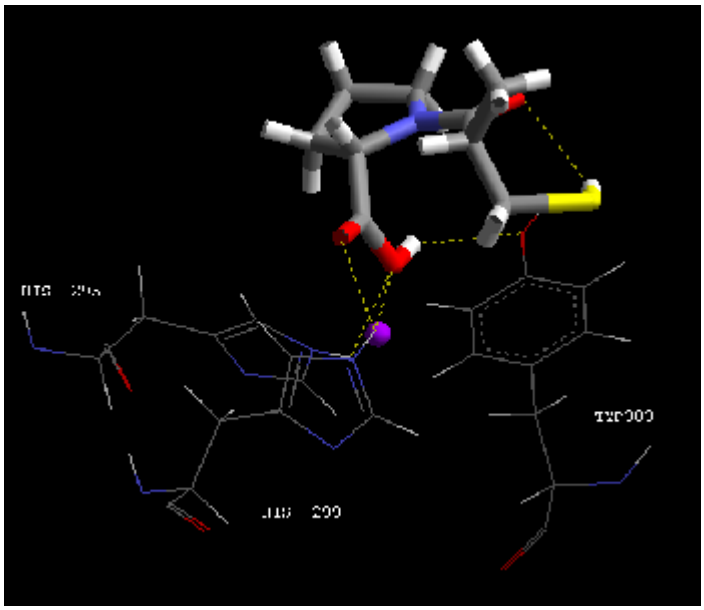

Illustration 10

Table 1.

Results of Pharmacophore hypothesis generated using training set against LTA4H inhibitors

HYPOTHESIS NUMBER	TOTAL COST	DIFFERENCE OF TOTAL COST AND NULL COST	RMS DEVIATION	FEATURES	TEST SET CORRELATION
1	105.148	53.62	1.0867	HA, HA, HPAr	0.920983
2	106.227	52.541	1.14147	HA, HD, HPAr	0.912111
3	108.264	50.504	1.24082	HA, HD, HPAr	0.8946057
4	108.92	49.848	1.26316	HA, HD, HPAr	0.890624
5	110.013	48.755	1.28036	HA, HD, HPAr	0.887981
6	111.13	47.638	1.34754	HA, HD, HPAr	0.87428
7	111.22	47.548	1.34916	HA, HD, HPAr	0.873982
8	111.81	46.958	1.35583	HA, HD, HPAr, HPAli	0.872958
9	113.49	45.278	1.42164	HA, HD, HPAr	0.85897
10	113.528	45.24	1.40374	HA, HD, HPAr	0.863351

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